

Ex-6

# THE LEUCOCYTE ANTIGEN *FactsBook*

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## Section II THE LEUCOCYTE ANTIGENS

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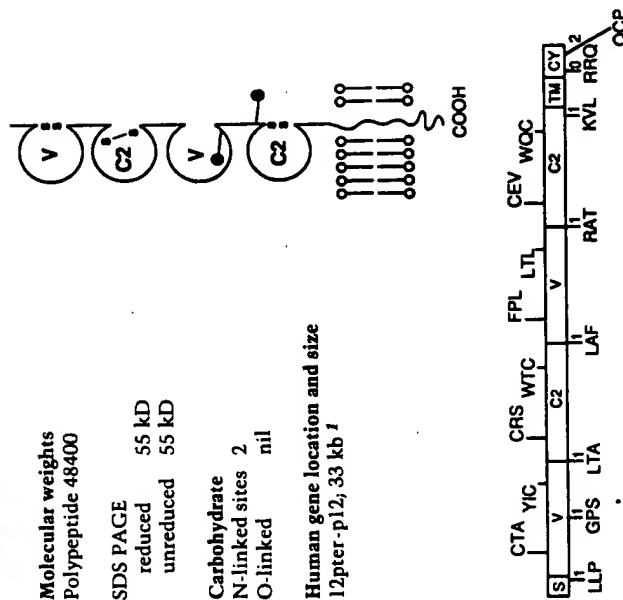
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Chatham, Kent

**Molecular weights**  
Polypeptide 48400

**SDS PAGE**  
reduced 55 kD  
unreduced 55 kD

**Carbohydrate**  
N-linked sites 2  
O-linked nil

**Human gene location and size**  
12pter-p12, 33 kb<sup>1</sup>



### Tissue distribution

CD4 is expressed on most thymocytes and approximately two thirds of peripheral blood T cells, which constitute the CD8 negative cells<sup>2</sup>. In human and rat but not in mouse, CD4 is expressed on monocytes and macrophages<sup>2</sup>.

### Structure

The extracellular domain is made up of four IgSF domains. The structures of the amino terminal two domains have been determined by X-ray crystallography, confirming that they are Ig-like<sup>3,4</sup>. Domain 2 is characterized by an unusual disulphide within one beta sheet and domain 3 lacks a disulphide in the position conserved in most IgSF domains. Cat CD4 shows some unusual features with 17 residues inserted between domains 1 and 2<sup>5</sup>. There is an additional Cys in domain 1 and the Cys in the unusual  $\beta$  strand C position in domain 2 is replaced with a Trp and there is an extra Cys in the  $\beta$  strand F<sup>5</sup>. The position of the NH<sub>2</sub>-terminus has been established for the rat homologue<sup>6</sup>.

### Function

CD4 is an accessory molecule in the recognition of foreign antigens in association with MHC Class II antigens by T cells<sup>2</sup>. MAbs against CD4

inhibit T cell functions *in vivo* and *in vitro*<sup>2</sup>. The cytoplasmic domain of CD4 is phosphorylated at Ser residues 408, 415, 431 (see below) when T cells are activated by antigen or phorbol esters<sup>7</sup>. The cytoplasmic domain interacts with a lymphocyte-specific tyrosine kinase called p56<sup>lck</sup> through a motif shown below<sup>8</sup>. CD4 is a receptor for HIV-1 (AIDS virus) and the binding of the viral gp120 protein is to a region of the amino terminal domain<sup>3,4</sup>.

### Comments

CD4 shows particularly close similarities in overall structure to the LAG-3 protein [see page 342].

### Motifs involved in CD4 function

p56<sup>lck</sup> recognition site (underlined) and Ser residues phosphorylated (in bold)  
RRQAE RMSOI KRLLSEKKTC QCPHRFQKTC SPI (433)

### Database accession numbers

	PIR	SWISSPROT	EMBL/GENBANK	REFERENCE
Human	A02109	P01730	M12807	2
Rat	A27449	P05540	M15768	6
Mouse	A02110	P06332	M13816	2

### Amino acid sequence of human CD4

-1					
MMHGRGVPFRHL	LLVLQLALLP	AAT06			
KKVVLGKKG	TVELTCTASQ	KKSIQFHWKN	SNQIKILGNQ	GSFLTQGPSK	50
LNDRADRRS	LWQGNFPLI	TKNLKIEDSD	TYICEVEDQK	EEVQLLVFGL	100
TANSDTHLQ	QSLTLTLES	PPGSSPSVQC	RSRGRKNIQG	GKTLVSQLE	150
LQSGTWTCT	VLQOKKVEF	KIDIVWLAFO	KASSIVYKKE	GEQVEFFSPL	200
AFTVEKLTGS	GELWQAEARA	SSSSSWITFD	LKNKEVSVKR	VTQDPKLQMG	250
KKPLHLPLHTLP	QALPOYAGSG	NLYLALEAKT	GKLHDEVLV	VMRATQLOKN	300
LTCEWVGPTS	PKMLSLKLE	NKEAKVSKRE	KAVWLNPEA	GNWQCLLSDS	350
GOVLLESNIK	VLPTWSTPVQ	PHALIVLGGV	AGLLLFILG	IFFCYRCRHR	400
RRQAE RMSOI	KRLLSEKKTC	QCPHRFQKTC	SPI		433

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# CD28

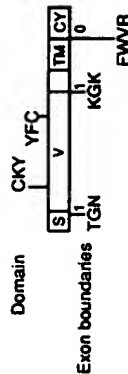
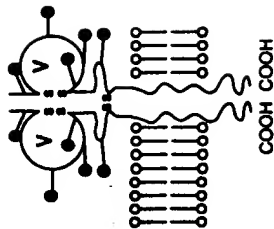
Tp44

Molecular weights  
Polypeptide 23085

SDS PAGE  
reduced 44 kD  
unreduced 90 kD

Carbohydrate  
N-linked sites 5  
O-linked unknown

Human gene location and size  
2q33-q34, 36 kb <sup>1</sup>



## Tissue distribution

CD28 is expressed on most T lineage cells and plasma cells <sup>2</sup>. Mature thymocytes have higher levels of CD28 than the immature cells and among peripheral T cells, 95% of CD4<sup>+</sup> cells and 50% of CD8<sup>+</sup> cells are positive <sup>2</sup>. Activation of T cells leads to enhanced CD28 expression <sup>2</sup>.

## Structure

CD28 is a member of the IgSF and is expressed as a disulphide-linked homodimer <sup>2,3</sup>. Human and mouse CD28 are 68% identical at the amino acid level <sup>4</sup>. CD28 is particularly similar to CTLA-4 with which it shares a ligand and probably a common ancestor in evolution <sup>4</sup>.

## Function

The ligand for CD28 is B7 <sup>5,6</sup> which is expressed on activated B cells, suggesting an important role for CD28 in the interaction between T and B cells. Activation of T cells via CD28 has provided evidence for a CD28 signalling pathway which involves stabilization of cytokine mRNA levels and is separate from that used by the TcR-CD3 complex <sup>2,7</sup>.

## Database accession numbers

PIR	SWISSPROT	EMBL/GENBANK	REFERENCE
Human	P10747	J02988	3
Mouse		M34563	4
Rat		X55288	8

## Amino acid sequence of human CD28

MLRLLALNL FPSIQVTG  
 HKILVKQSPM LVAYDNAVNL SCKYSYNLFS REFRASLHKG LOSAVEVCVW  
 YGNYSQQLQV YSKTGFNCDG KLGHSVTFF LQNLVYVOTD IYFKIEVWY  
 PPYLDNEKS NGTITHYKKG HLCPSPLFPG PSKPEWLVV VGGVLACYSL  
 LTVIAELIEH VRKRSRLH SDYNNMTPRR PGTRKKHYQP YAPPRDFAAY  
 RS

## References

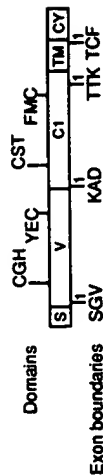
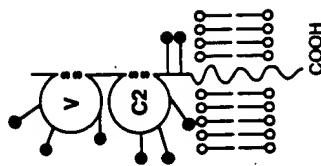
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**Molecular weights**  
Polypeptides 30048

**SDS PAGE**  
reduced 60 kD  
unreduced 60 kD

**Carbohydrate**  
N-linked sites 8  
O-linked unknown

**Human gene location and size**  
3q13.3-3q21, 32 kb <sup>1</sup>



## Tissue distribution

Present on a subset of B cells *in vivo* and the majority of B cells activated *in vitro*. Red blood cells, granulocytes, monocytes, resting or activated T cells, thymocytes and platelets do not express B7 <sup>2</sup>. The antigen is expressed by HTLV-1 transformed T cells <sup>3</sup>.

## Structure

The extracellular domain contains two IgSF domains which are highly glycosylated <sup>4</sup>. The sequence of the transmembrane domain is unusual containing 3 cysteine residues that might be covalently modified or participate in intermolecular interactions <sup>4</sup> although there is no evidence for this. The cytoplasmic domain has a preponderance [9/19] of arginine residues and contains a potential site for calmodulin-dependent phosphorylation [RRES] <sup>4</sup>.

## Function

B7 is the ligand for the CD28 <sup>5</sup> and CTLA-4 <sup>6</sup> glycoproteins. Cells transfected with either human <sup>7</sup> or murine <sup>8</sup> B7 genes supply co-stimulatory signals to human T cells, suggesting that the CD28 binding site is conserved <sup>8</sup>. The antigen is not expressed on resting B cells but is strongly upregulated on B cells activated with a variety of agents, including the Epstein-Barr virus <sup>2</sup>, cross-linking anti-IgM <sup>2</sup>, anti-CD45 and anti-MHC Class II mAbs <sup>9</sup>, IL2 and IL4 <sup>10</sup>. MAbs to B7 block the differentiation of B cells into antibody secreting cells <sup>11</sup> and the alloactivation of T cells <sup>9</sup>.

## Comments

This antigen is not related to a mouse antigen called B7 and to avoid confusion the latter is being called B7[2].

## Database accession numbers

Human M27533  
Mouse X60958

## Amino acid sequence of human B7

MGHTRRQGTSPSKCPYLNNFFQLLVLA  
GLSHFCSGVIHVTKEVKEVATLSCGHNVSEELAQTRIYWQEKKKVLTLM 50  
MSGDMIMPEYKNTIFDITNLSIVILALRPSDEGTVECVLKYKDAF 100  
KREHLAEVTLVYKADFTPTISDFEIPSTNIRRIICSTSGGFPEPHLSWL 150  
ENGEELNAINITVSQDPETE·LYAVSSGLDFNMTHNSFMC LIRYGHLRVN 200  
QTFNNTTKQEHFQDNLPSWAILISYNGJFVICCLTYCFAPRCRERRR 250  
NERLRRESVRPV 262

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